

सही करने की हिम्मत उसी में आती है
जो गलती करने से नहीं डरते हैं।

CSIR NET – Life Science

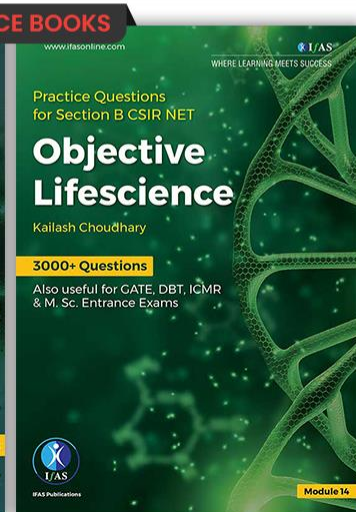
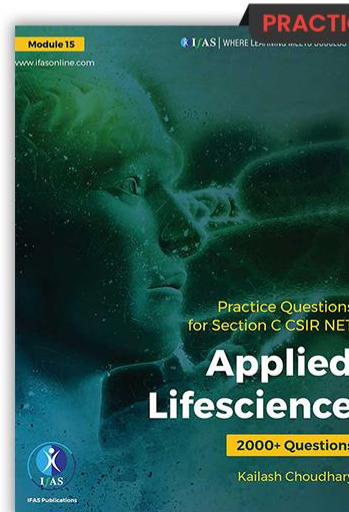
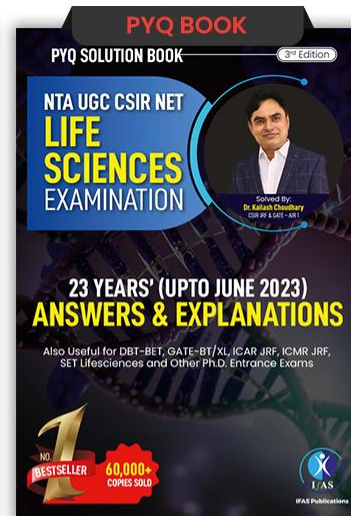
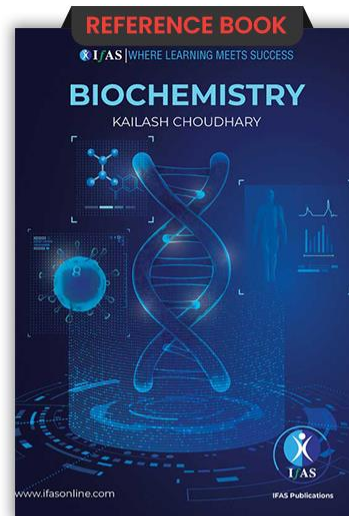
Unit 1: Biochemistry

08

Conformation and stability of
Nucleic Acids








Order Online and Get
Free Delivery Across India



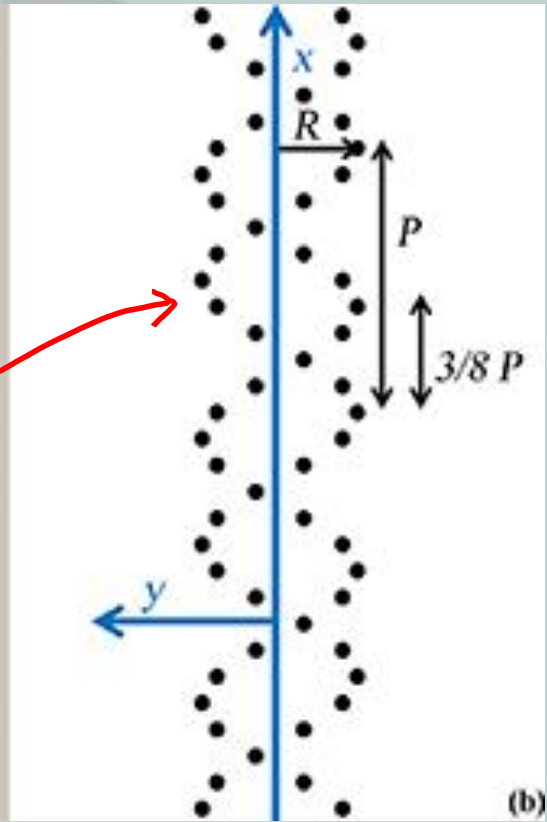
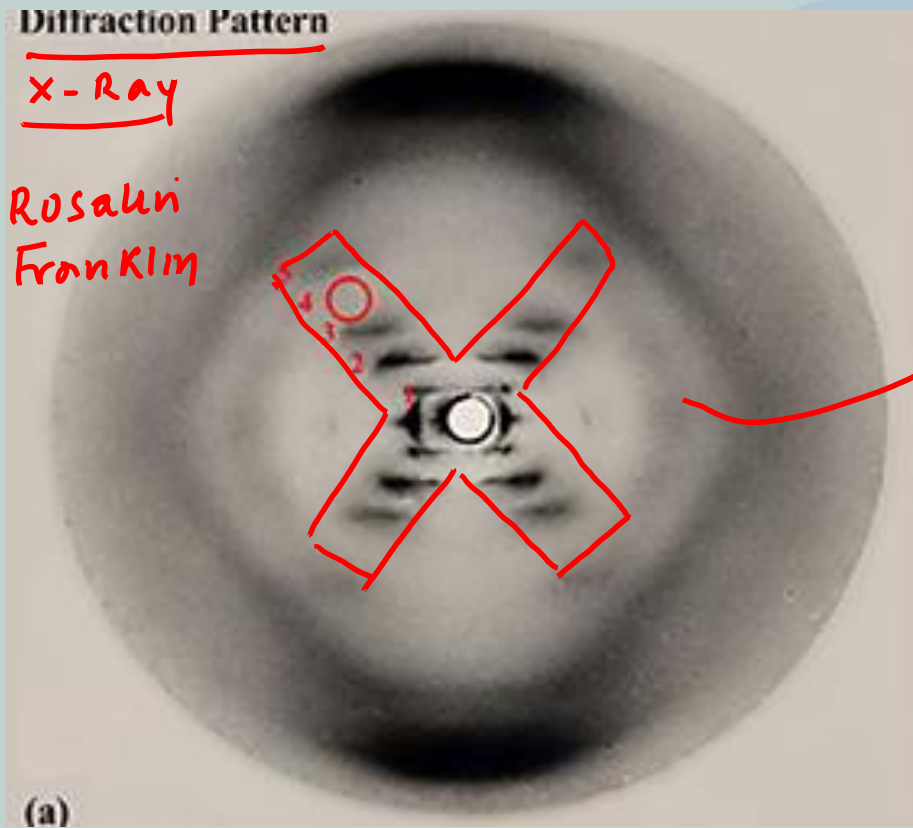


Points to be covered in this Lecture

-  Chargaff Rule
-  B-form of DNA
-  A-form of DNA
-  Z-form of DNA
-  Stability of DNA
-  Renaturation of DNA
-  t-RNA
-  mi-RNA



DNA-BASIC STRUCTURE – B form



molecular model

↓

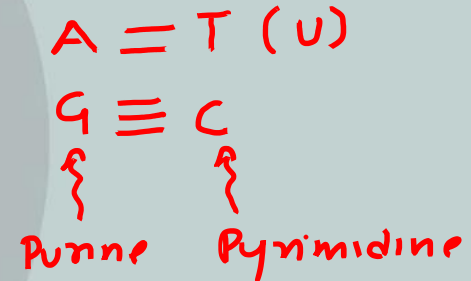
watson & crick



I. Chargaff's Rules: Applicable for Double stranded DNA or RNA

The number of adenosine residues is equal to the number of thymidine residues (that is, A = T), and the number of guanosine residues is equal to the number of cytidine residues (G = C).

$$\begin{aligned}
 &\text{Purine} = \text{Pyrimidines} \\
 \checkmark \quad A + G &= T + C \\
 \checkmark \quad A + C &= T + G \\
 A + T &\neq G + C
 \end{aligned}$$



Nature of Genome can be identified

If it has A, T, G and C : DNA

If it has A, U, G and C : RNA

✓ If A = T (U) and G = C : Double stranded ✓

If A not equal to T (U) and G not equal C : Single stranded

<u>A</u>	<u>T</u>	<u>G</u>	<u>C</u>	<u>U</u>	Nature
20	20	30	30	0	<u>DS DNA</u>
<u>20</u>	-	<u>30</u>	<u>30</u>	<u>20</u>	<u>DS RNA</u>
<u>25</u>	<u>35</u>	<u>15</u>	<u>35</u>	0	<u>SS DNA</u>
18	0	36	24	22	<u>SS RNA</u>



Base composition can be determined for only double stranded DNA or RNA

Suppose it is given that A is 22%, what will % composition of rest of bases?

- If A is 22 % means T must be also 22% = 44.%. A T
- Rest 56 % must be G and C which must be equal = 56.%. G C
- So 28 % must be G and 28 % must be C



Apply your Mind

1. On sequence analysis of a double stranded DNA, the results showed the content of cytosine, C was 20%. What is the amount of A?

(1) 20%

☒ (2) 30%

(3) 50%

(4) 60%

$$\begin{array}{lcl} \text{C} & 20\% & \text{G} 20\% = 40\% \\ \text{A} & 30\% & \text{T} 30\% = 60\% \end{array}$$

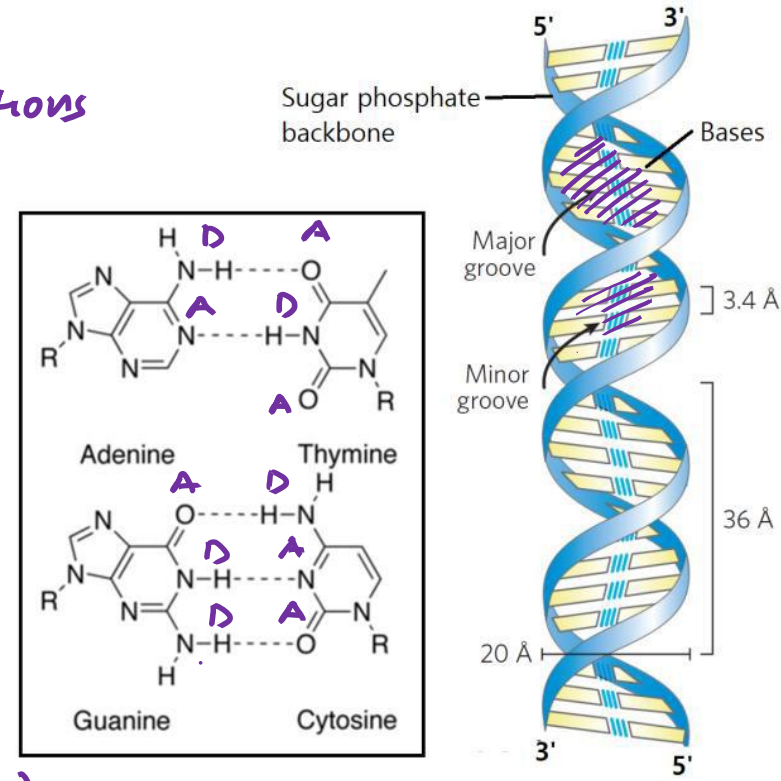
A 30%



Watson and Crick's Double Helix: B-DNA

most common form under physiological conditions

- ✓ 1. Double stranded
- ✓ 2. Antiparallel
- ✓ 3. Helical
- ✓ 4. Right handed
- ✓ 5. Diameter = 20 Å. (2 nm) Divide by 10
- ✓ 6. Hydrogen bonding A with T and G with C
- ✓ 7. The planes of base pairs are perpendicular to the helix axis. $\Delta^0 = 10^{-10} \text{ m}$
 $\text{nm} = 10^{-9} \text{ m}$
- ✓ 8. 10.5 base pairs per turn (~10 bp)
- ✓ 9. The helix rotates 36° per base pair.
- ✓ 10. Helix height 36 Å. or 3.6 nm
- ✓ 11. Distance between two base pairs 3.4 Å. (0.34 nm)
- ✓ 12. Major and minor grooves.





AT and GC Hydrogen bonding

(T)

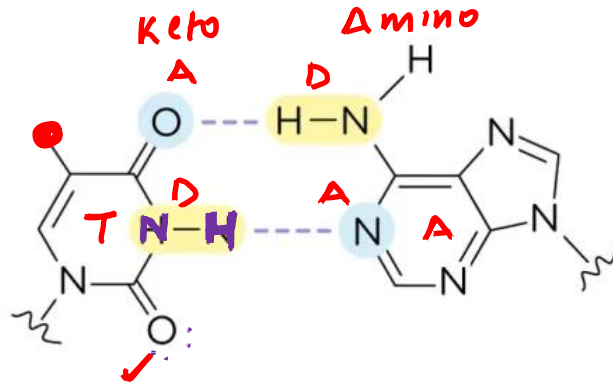
Acceptor ~~→~~ Acceptor
Donor ~~→~~ Donor
Acceptor Donor

(G)

T ≠ G
C ≠ A

Hydrogen bond acceptors

Hydrogen bond donors

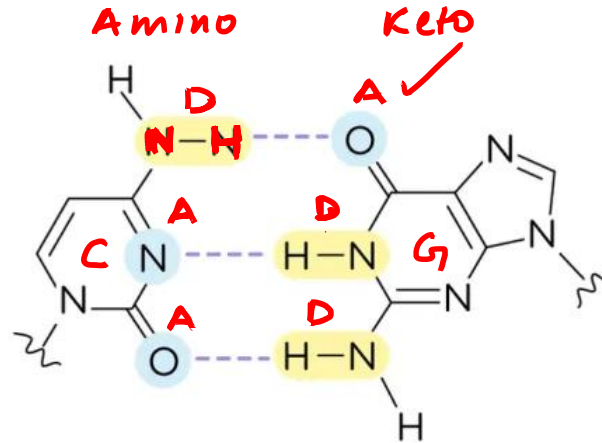


Thymine
or Uracil

1 donor
1 acceptor

Adenine

1 donor
1 acceptor



Cytosine

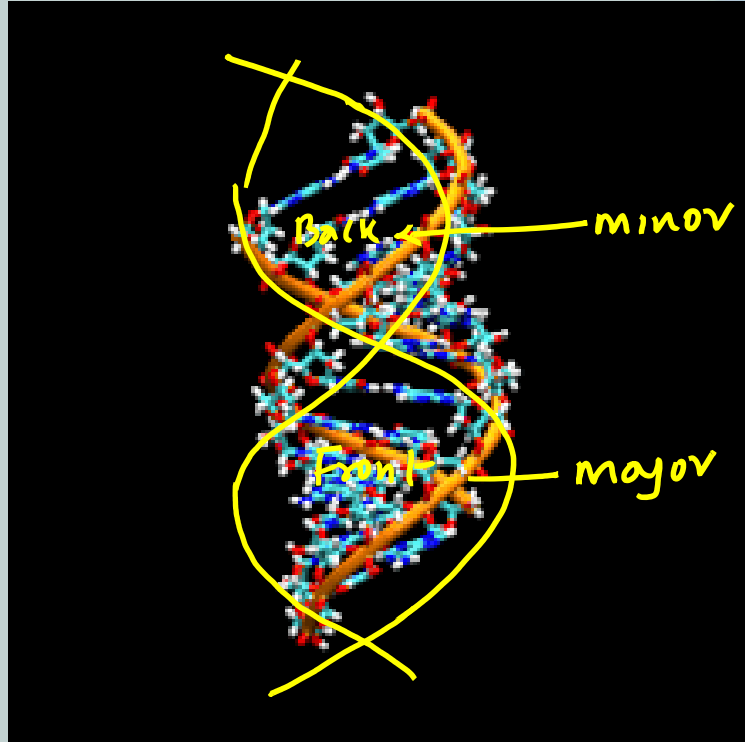
1 donor
2 acceptor

Guanine

2 donor
1 acceptor



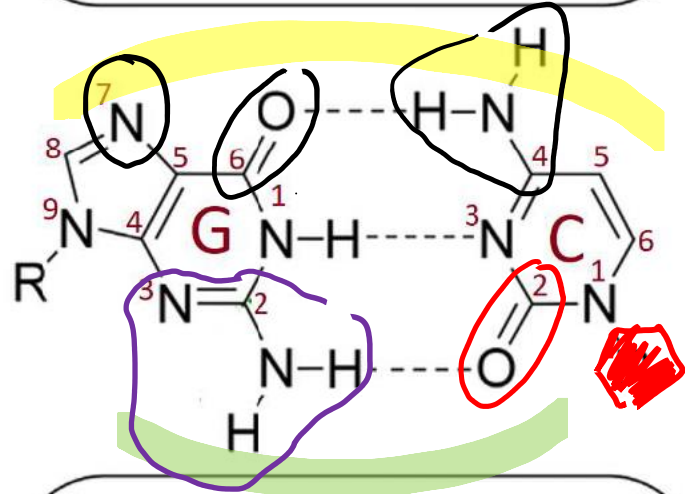
Major and Minor Groove : crevice from where bases are visible & proteins can bind



major : Front face is visible which is wide — more bases are seen
→ most of DNA binding proteins interacts in major groove

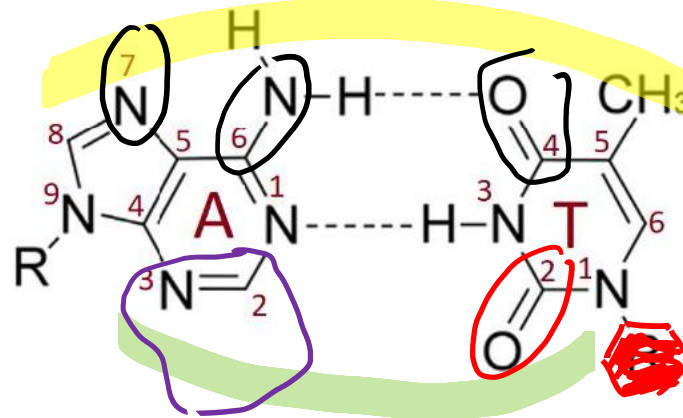
minor : Back of bases is visible
Narrow, limited bases are visible
Few protein interact to minor groove
eg TBP, Histone, AT hook

Major Groove



Minor Groove

Major Groove



Minor Groove

Imp

Major Groove

Purine: C₆ & N₇

Pyrimidine: C₄
(Keto/Amino)

Minor groove

Purine: C₂ & N₃

Pyrimidine: C₂ (Keto)



Size of DNA Molecules in meters

* Distance betⁿ 2 nucleotide pair = $3.4 \text{ \AA} = 3.4 \times 10^{-10} \text{ m}$
 $= 0.34 \text{ nm} = \underline{0.34 \times 10^{-9} \text{ m}}$

Human genome length :→

↳ Size : $3 \times 10^9 \text{ bp (np)}$
 $= 3 \times 10^9 \times 0.34 \times 10^{-9} \text{ m}$
 $= 3 \times 0.34 \text{ m}$
 $= 1.02 \text{ meter}$

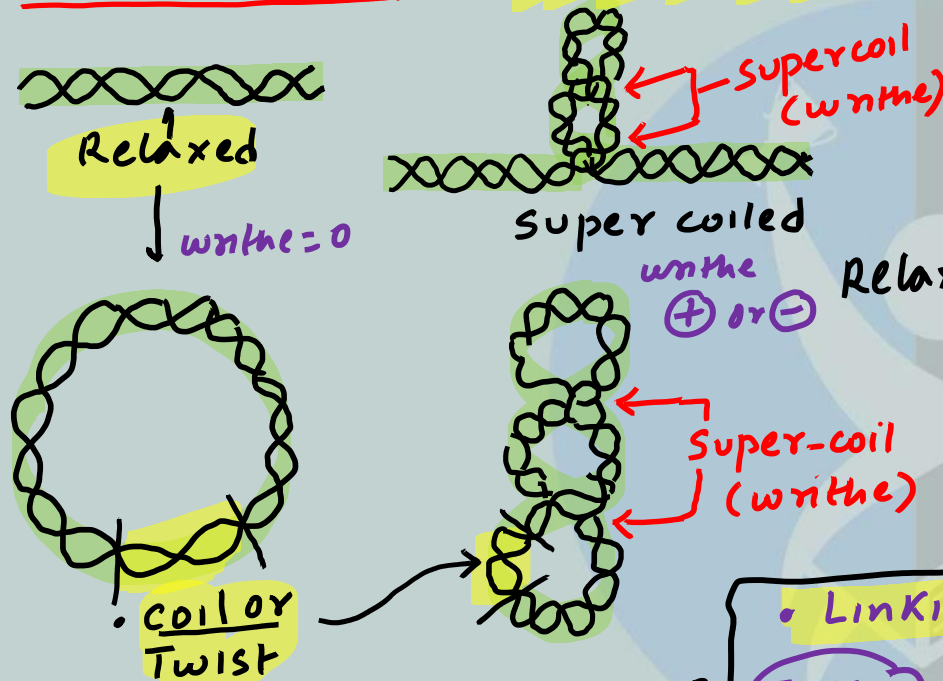
→ 23 Fragment
 Largest Fragment = Chromosome 1

Total length of DNA in nucleus of human somatic cell?

↳ Diploid → Two copies of Genome → $2n = 46$ fragments
 $= 2 \times 1.02 \text{ m} = 2.04 \text{ meter}$



Topological forms and Linking number



- After every 10 bp or 34\AA

$$LK = 500 + 10 = 510$$

• Linking Number (LK) = Twist \pm writhe

• 5 Kbp plasmid with 10 positive supercoil

Linking number? $\frac{5000\text{bp}}{10\text{bp}} = 500$ (Twist)

$LK = Tw + wr =$

Positive Supercoiling

- overwound condition, when extra twist is introduced
- Ahead of replication / transcription fork or bubble

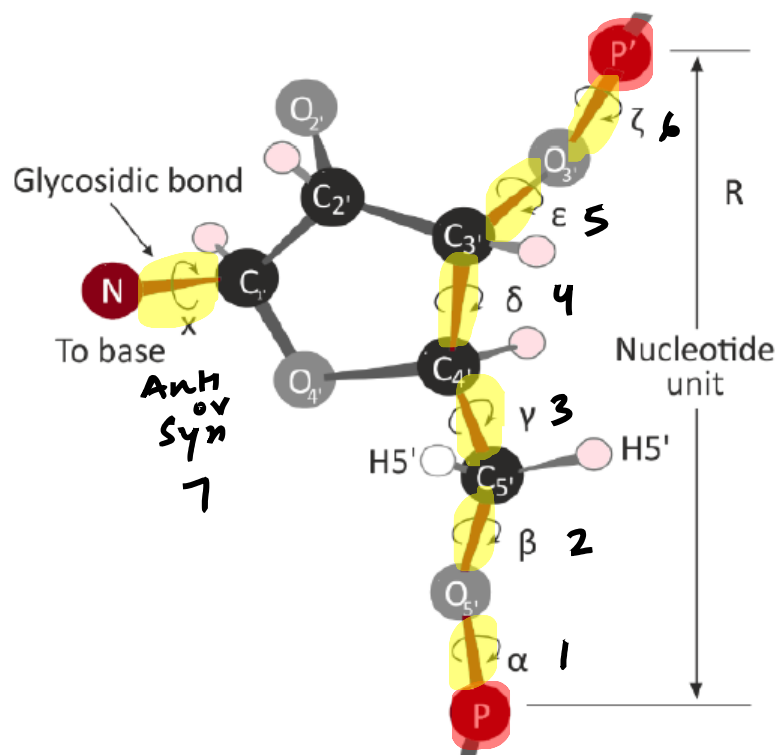


Negative supercoiling

- underwound condition
- Lesser twist

Relaxed **Topoisomerase**

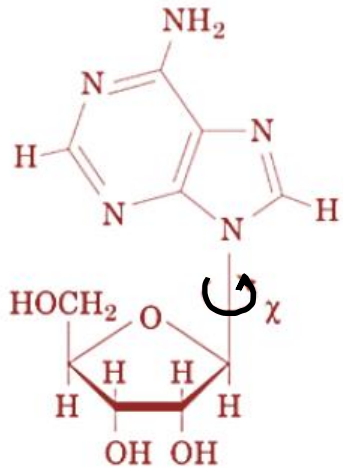
CONFORMATION OF DNA- A, B and Z



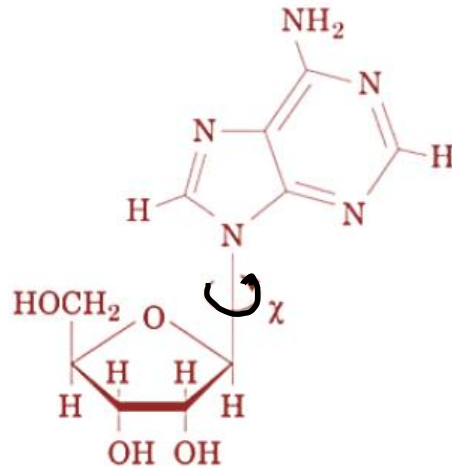
Atleast 7 bonds where rotation can lead to different conformation — A, B, Z form



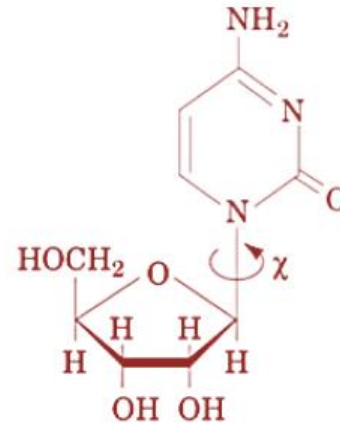
I. Torsion Angles about Glycosidic Bonds: Anti or Syn



Syn-Adenosine



Anti-adenosine



Anti-cytidine

A or B-form

✓ Anti is more favorable
Less steric hindrance

Rare

Syn is seen only in purines
of Z-form of DNA

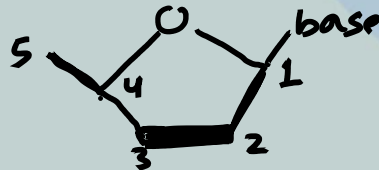
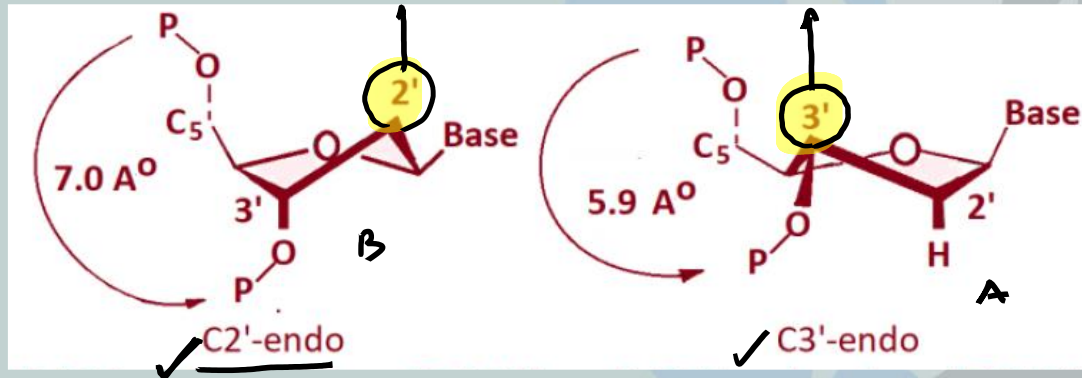


Sugar Puckering

- Four of the five ring atoms are coplanar
- 5th atom is out of the plane in a **half-chair** conformation.

Endo- conformation:

If **out of plane atom** is the **same side of the ring**



Imp :

C2' endo:

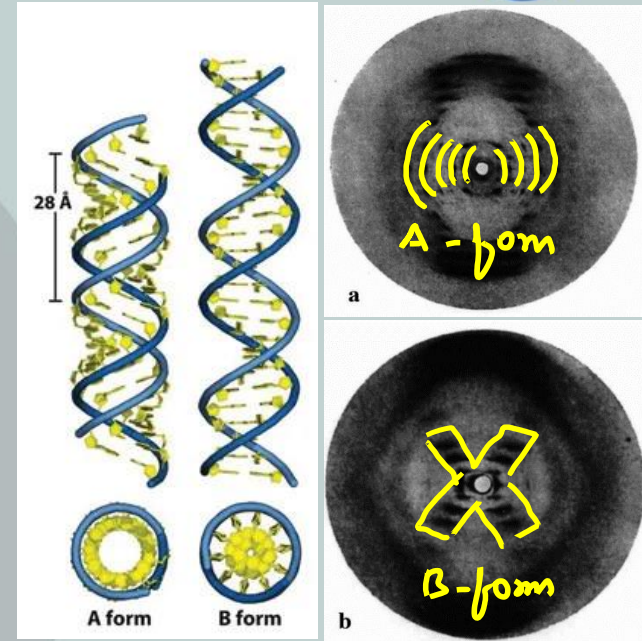
B-form of DNA
Z-form- Pyrimidine

C3' endo:

A-form of DNA ✓
Z-form- Purines ✓

A-form of DNA

- ✓ Dehydrated condition: RH is less than 75%
- ✓ Not seen in biological system (Imp)
- ✓ Right handed
- ✓ Diameter: 26 \AA (2.6 nm)
- ✓ Base pairs per helical turn: 11 (11.6 bp)
- ✓ Sugar pucker: C3' endo
- ✓ Glycosyl bond conformation: Anti
- ✓ Major groove: Narrow and deep
- ✓ Minor groove: Shallow and wide



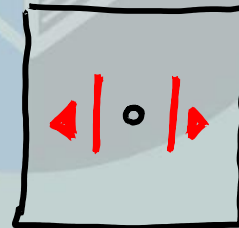
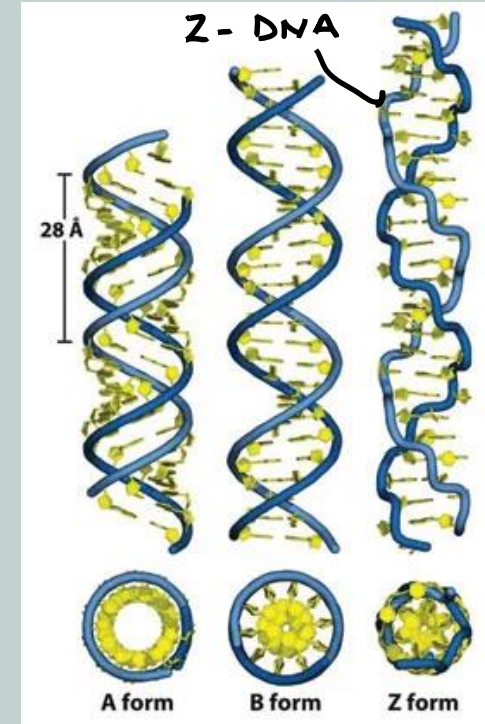
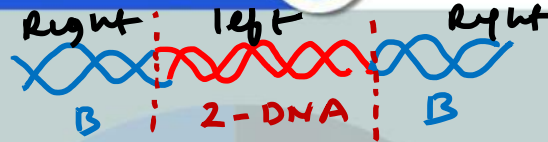
Double-helical DNA: RNA hybrids and DS RNA have an A-like conformation

DNA - RNA and RNA - RNA



Z-form (Zig-Zag) of DNA

- DNA with alternate Pyrimidine-Purine Sequence
- GpCpGpCpGpCpGpC Salt = \uparrow , -ve supercoiling = \uparrow , methylation = \uparrow
- ✓ Left handed
- ✓ Diameter: 18 Å (1.8 nm)
- ✓ Base pairs per helical turn : 12
- Sugar pucker: C2' endo for Pyrimidine and C3' endo for Purine
- ✓ Glycosyl bond : Anti for pyrimidines; syn for purines
- Major groove: Shallow and wide (almost absent)
- Minor groove: Narrow and deep
very





	A-form	B-form	Z-form
Helical sense	<u>Right handed</u>	<u>Right</u>	<u>Left handed</u>
Diameter	<u>26 Å</u>	<u>20 Å</u>	<u>18 Å</u>
Base pairs per helical turn	11 <u>11.6</u>	<u>10.5</u>	<u>12</u>
Sugar pucker conformation	<u>C-3' endo</u>	<u>C-2' endo</u>	<u>C-2' endo for pyrimidines;</u> <u>C-3' endo for purines</u>
Glycosyl bond conformation	<u>Anti</u>	<u>Anti</u>	<u>Anti for pyrimidines;</u> <u>syn for purines</u>
Major groove	<u>Narrow and deep</u>	<u>Wide and intermediate depth</u>	<u>Shallow and flat (absent)</u> <u>very</u>
Minor groove	<u>Shallow and wide</u>	<u>Narrow and intermediate depth</u>	<u>Deep and narrow</u>

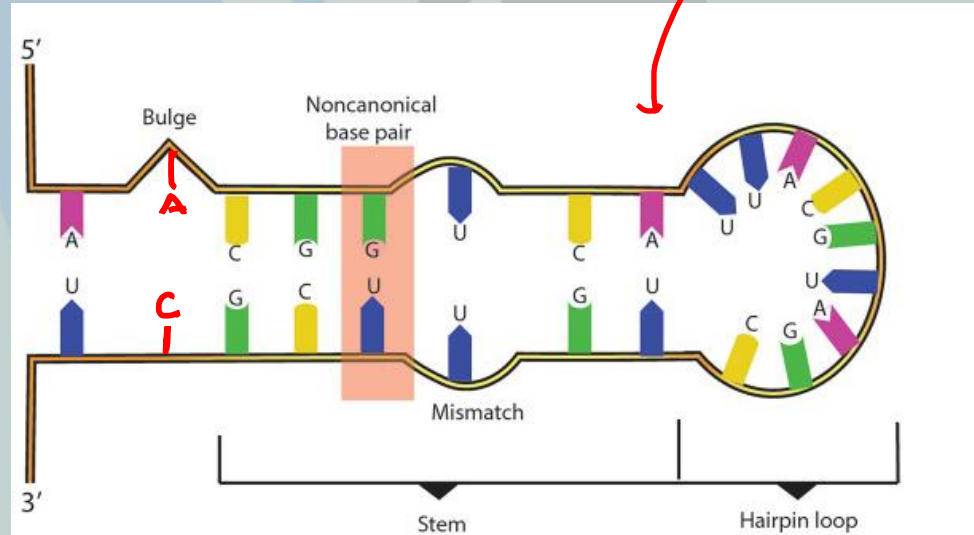
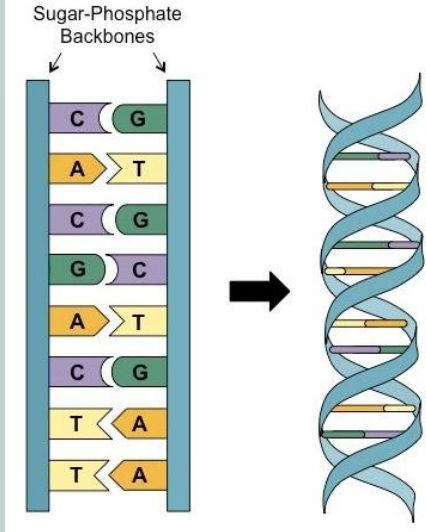
Stabilizing Interactions in DNA

Hydrogen Bonds:



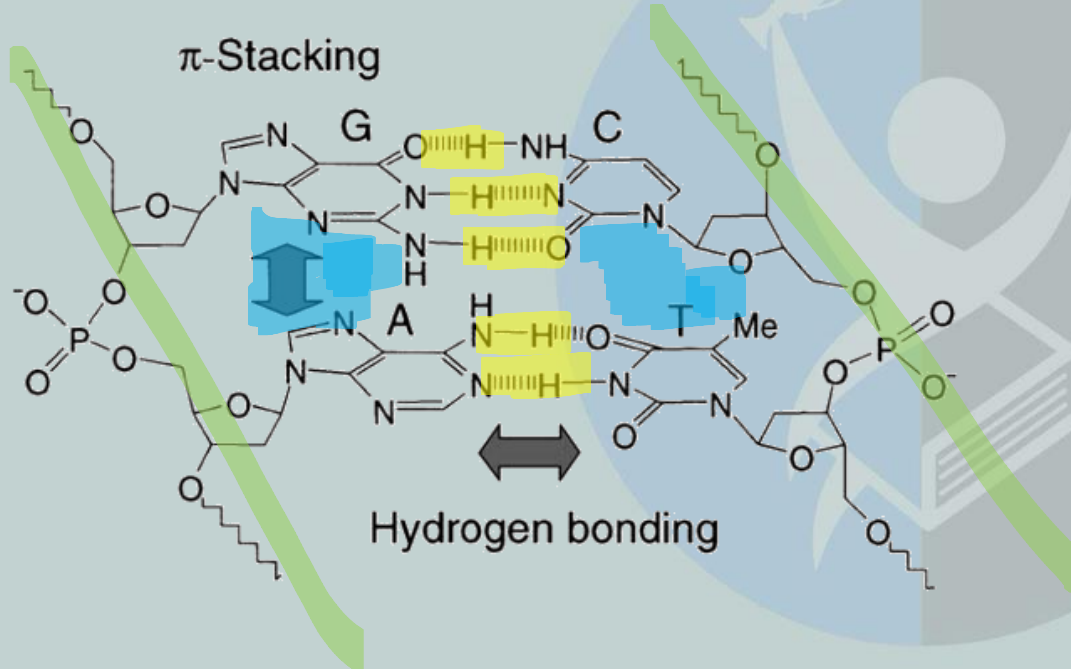
✓ Double stranded DNA: More GC content more stable structure

Single stranded DNA: more complementary bases at 5' and 3' ends more stable 2^o structure



Base Stacking:

- Hydrophobic and Vander Waals interactions between bases in the same strand
- More stability when purine or pyrimidines are adjacent



stacking ✓



X



Destabilisers

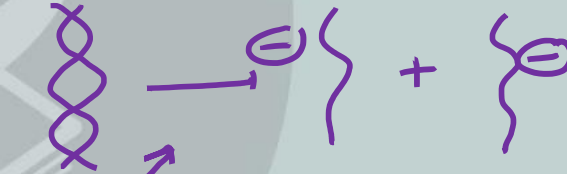
Ionic Interactions

- Repulsive due to phosphate ions
- ~~Magnesium ion~~ can neutralize the negative charges
monovalent cations (Na^+ , K^+ , NH_4^+)

Denaturing Conditions

- Urea
 - Formamide
 - High pH, *Low pH*
 - High temperature
 - DNA mismatch
 - Low ionic strength of salts
- Hydrogen bond Disrupt
- Lower than Physiological salt conc

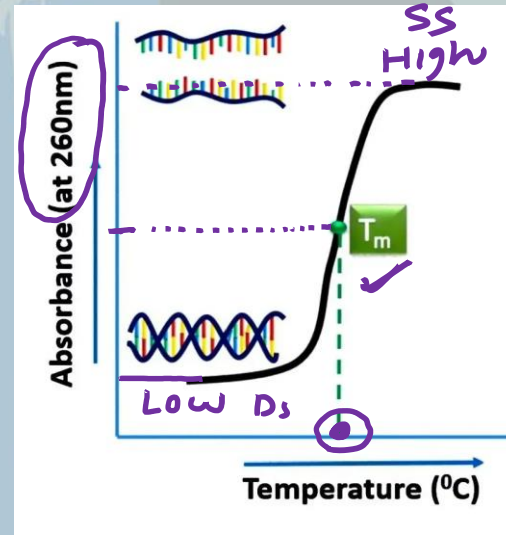
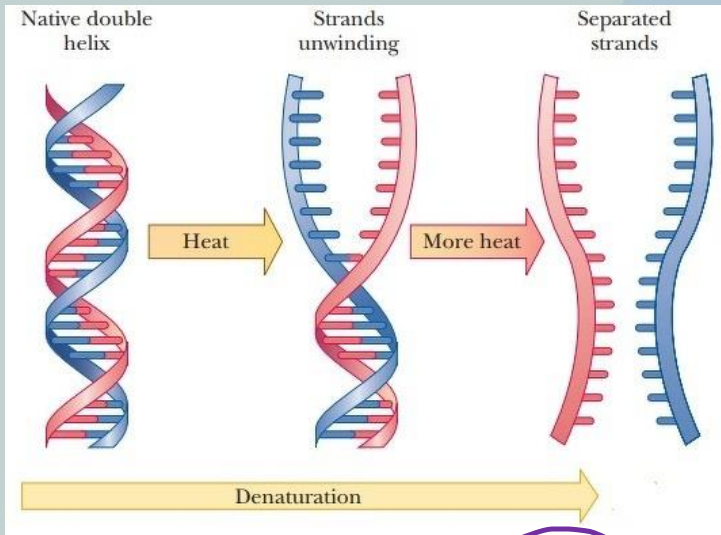
→ Phosphate group -ve charge will not be neutralize





Denaturation of DNA

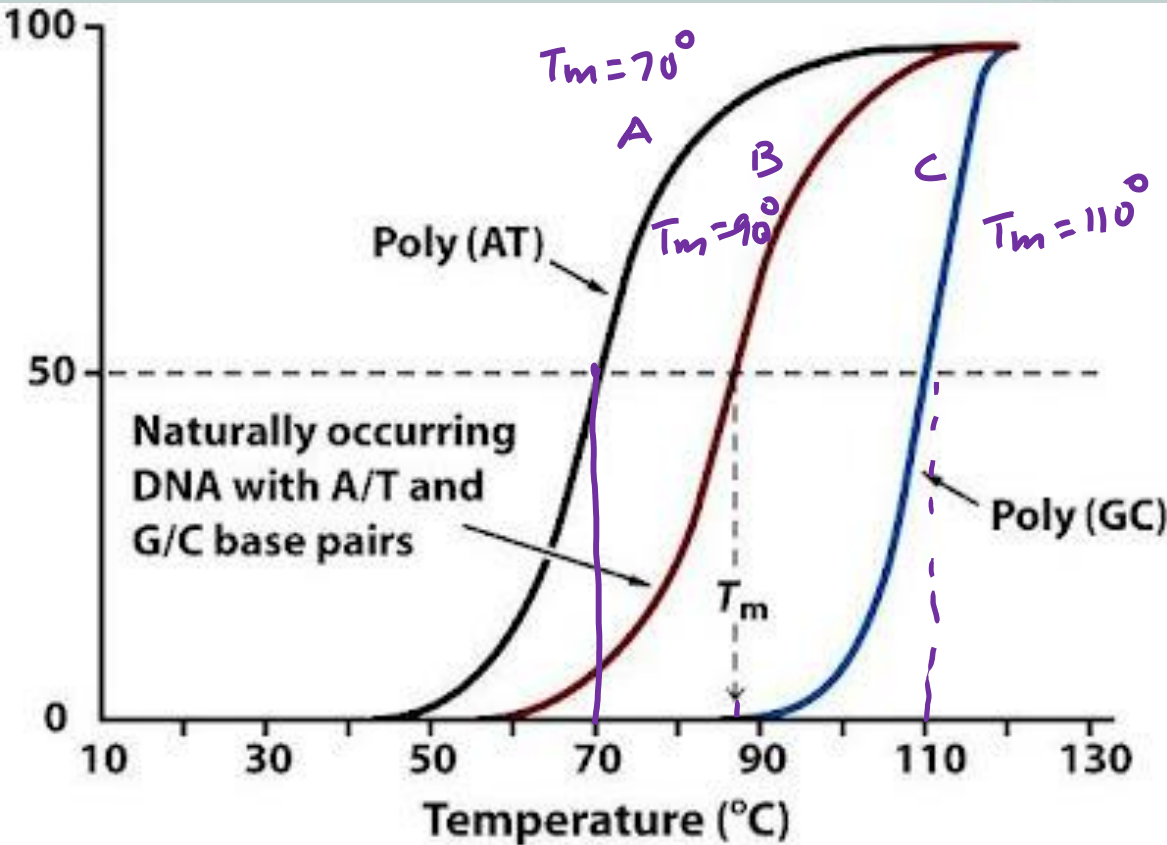
Process where the double strands of the DNA molecule separate into single strands



Hyperchromic Effect

As DNA denatures, its absorption of UV at 260 nm increases

Melting temperature (T_m): It is the temperature at which half of the DNA helix is denatured into single strands.

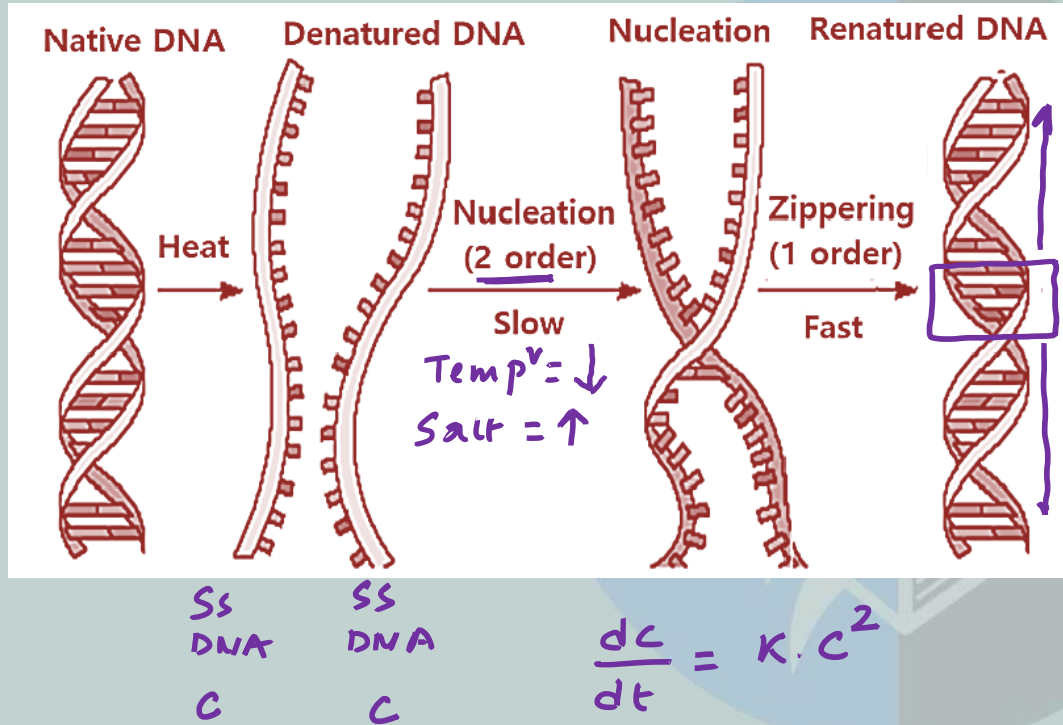


High T_m

- More GC, low AT
- More monovalent cations

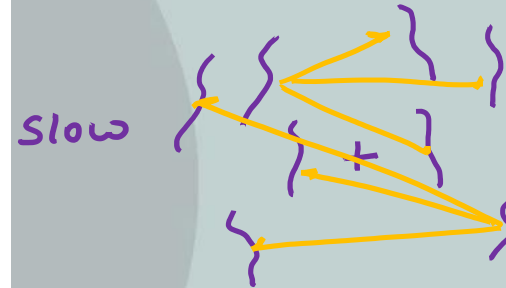
$T_m \propto$ GC content
 $T_m \propto$ monovalent cation
 (Na^+ , K^+ , NH_4^+)
 (ionic strength of salt)

DNA Renaturation



The rate of renaturation depends on

- Number of repeats = \uparrow fast





Renaturation depends on

- DNA concentration (repetitive sequences)
- Renaturation temperature
- Cation concentration Na^+ , K^+ , NH_4^+
- Viscosity of DNA

$$\underline{C_0} \times \underline{t} \times \text{buffer factor} \quad (L)$$

Fast Renaturation

- more repeats
- Low Temp^r
- High ionic strength of salt
- Low viscosity.

Cot = DNA conc. (mol/L) **x** renaturation time in sec **x** a buffer factor
(that accounts for the effect of cations on the speed of renaturation)

Cot/2, the Cot value where half of the DNA has ^{renatured} reannealed.



Total cot value of DNA

$Cot = \text{DNA Concentration (moles/L)} \times \text{renaturation time in seconds} \times \text{buffer factor}$

Nucleotide concentration = 0.050 M

Renaturation time = 344 sec

Buffer factor = 5.820

Cot value = $0.050 \times 344 \times 5.820 = \underline{100.000}$

$C_o \times t \times \text{buffer factor}$

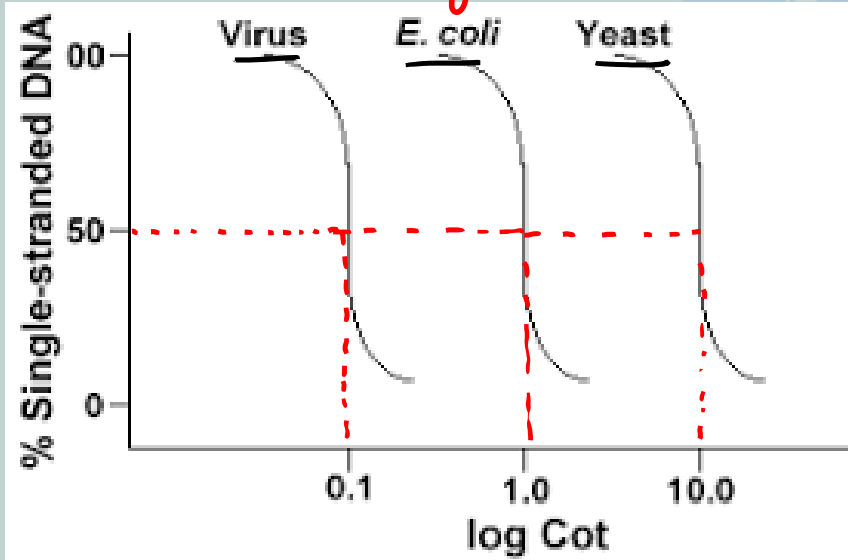
Cot Curve

X axis = $\log \text{Cot}$
Y axis = % of ss DNA

Curve depends on

1. the size or complexity of the genome

Nature of genome is same (unique sequence)



$\text{Cot}_{1/2} (\text{Yeast}) > \text{E. coli} > \text{virus}$
 $10^7 \text{ bp} > 10^6 \text{ bp} > 10^5 \text{ bp}$

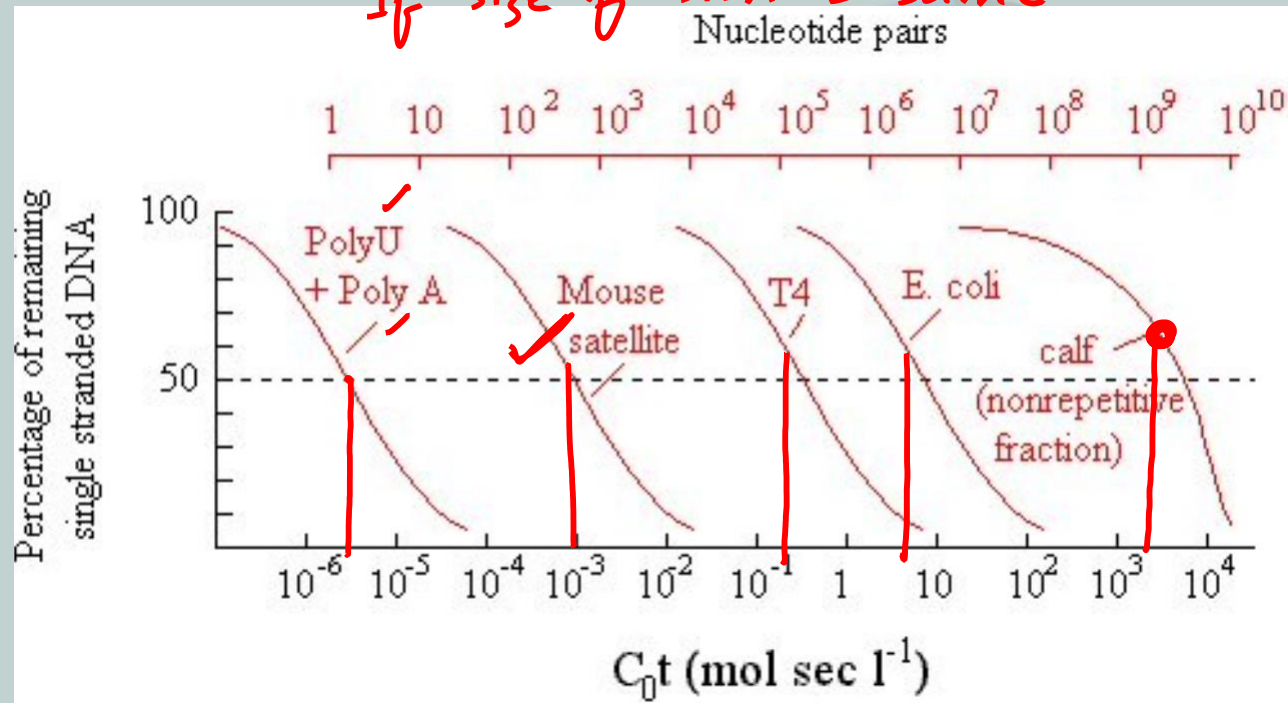
→ complexity of genome (small or large)

→ Nature of genome (Repeats or unique)



2. the amount of repetitive DNA within the genome

If size of DNA is same



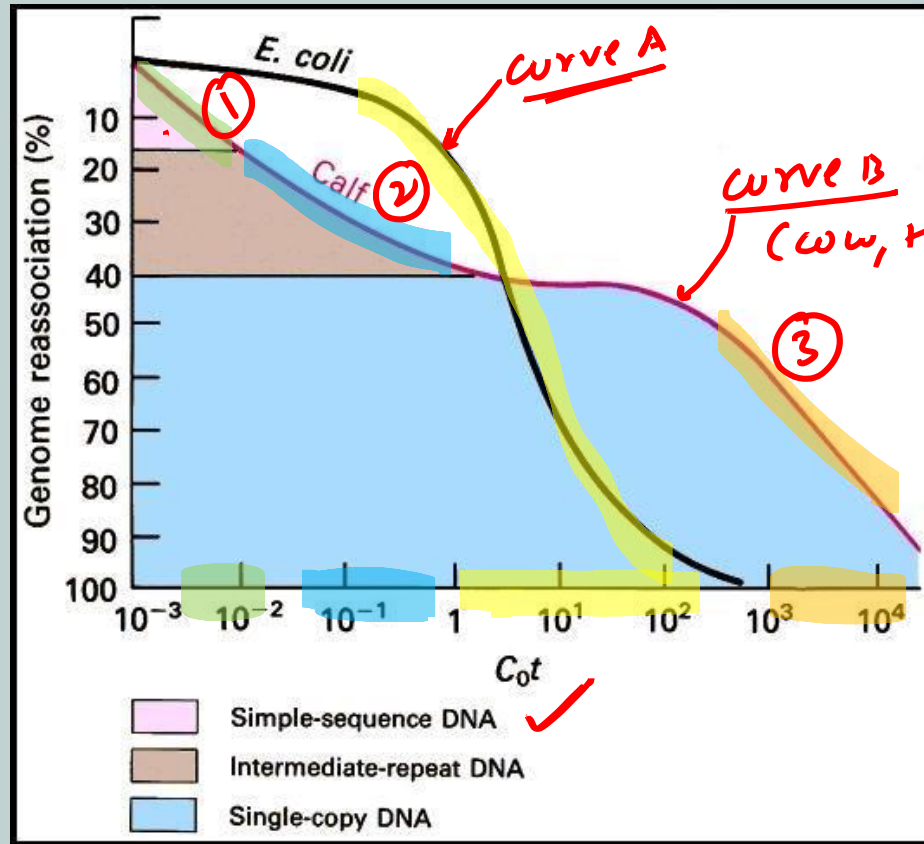
Low Cot value

- more repetitive DNA
- Poly A $\begin{matrix} A \\ A \\ A \\ A \end{matrix}$ $\begin{matrix} U \\ U \\ U \\ U \end{matrix}$ Poly U
- Satellite DNA

High Cot value

- Unique DNA (non-repetitive DNA)

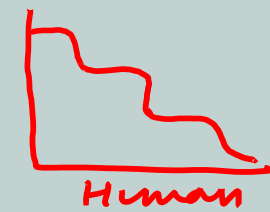
Prokaryotic and Eukaryotic Cot curve



✓ E. coli : unique DNA
& small genome

Cot value Calf / human

Low ① → Highly repetitive DNA (SSR or satellite)
medium ② → moderate repeats eg SINES and LINES
High ③ → unique DNA or single copy DNA





Cot value (in moles nucleotide liter⁻¹) of particular sequence

Fraction of sequence X Concentration (moles/lit)

$$\underline{\text{conc}}^v = 0.01 \text{ moles/lit}$$

$$\text{unique DNA (50\%)} = 0.5$$

$$\begin{aligned} \text{Cot value of unique DNA} &= 0.01 \times 0.5 \\ &= 0.005 \end{aligned}$$

Fraction is out of 2

$$\underline{10\%} \rightarrow \text{Fraction} = \underline{0.1}$$



Apply your mind:

Genome of an organism was analysed by Cot curve analysis. Highly repeated sequences represented 30% of the total genome fraction. The Cot value of the highly repeated sequence was found to be 0.001 moles nucleotide liter⁻¹. What would be the actual Cot value (in moles nucleotide liter⁻¹) of the highly repeated sequence?

(1) 0.003

(2) 0.001

✓ (3) 0.0003

(4) 0.007

$$= 0.001 \times \underline{0.3}$$

$$= 0.0003$$

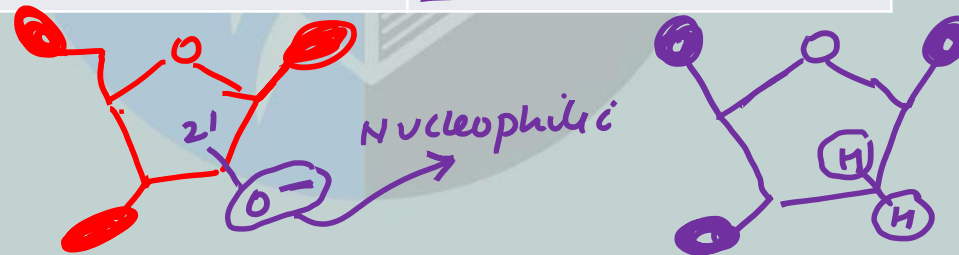
$$30\% = \frac{30}{100} = \underline{0.3}$$



Differences Between RNA and DNA

	RNA	DNA
Sugar Component	<u>Ribose</u> (2'-OH)	<u>2'-Deoxy Ribose</u>
Base Pairing	<u>Uracil=A</u>	<u>Thymine=A</u> (5-methyl uracil)
Structure	<u>Generally Single stranded</u>	<u>Generally double stranded</u>
Double stranded Structure	<u>Like A form of DNA</u>	<u>Generally B form</u> (Rare <u>Z form</u>)
Stability	<u>Low</u> (2'-OH act as nucleophile)	High
Functions	<u>Many</u>	<u>Genetic Material</u>

Help BER mechanism to identify U produced by deamination of cytosine





RNA diversity

SSRNA: TMV, RENVIRUS
DSRNA: RHEOVIRUS

Concⁿ: rRNA > tRNA > mRNA

Types: m-RNA > tRNA > rRNA
>1000 30-35 3-4

Type of RNA

mRNA (Messenger RNA)

Function

Serves as a template for protein synthesis

tRNA (Transfer RNA)

Brings amino acids to ribosomes during protein synthesis, recognizes codons

rRNA (Ribosomal RNA)

Structural and catalytic component of ribosomes

snRNA (Small Nuclear RNA)

Involved in RNA splicing, part of the spliceosome complex that processes pre-mRNA.

snoRNA (Small Nucleolar RNA)

Plays a role in chemical modifications of other rRNAs (rRNA processing)

miRNA (MicroRNA)

Regulates gene expression by binding to mRNA molecules and blocking their translation or causing degradation.

siRNA (Small Interfering RNA)

Involved in the RNA interference pathway, targeting specific mRNA for degradation.

piRNA (PIWI-interacting RNA)

Protects the genome integrity in germ cells by suppressing transposons.

lncRNA (Long Non-Coding RNA)

Involved in a variety of cellular processes, including chromatin modification, transcriptional regulation, and cell differentiation.

crRNA (CRISPR RNA)

Part of the CRISPR-Cas system, guiding Cas proteins to specific DNA sequences for cleavage in adaptive immune responses.

Guide RNA

→ RNA editing

scRNA

• Protein Targeting

Riboenzymes

• Catalytic RNA

Nucleolus

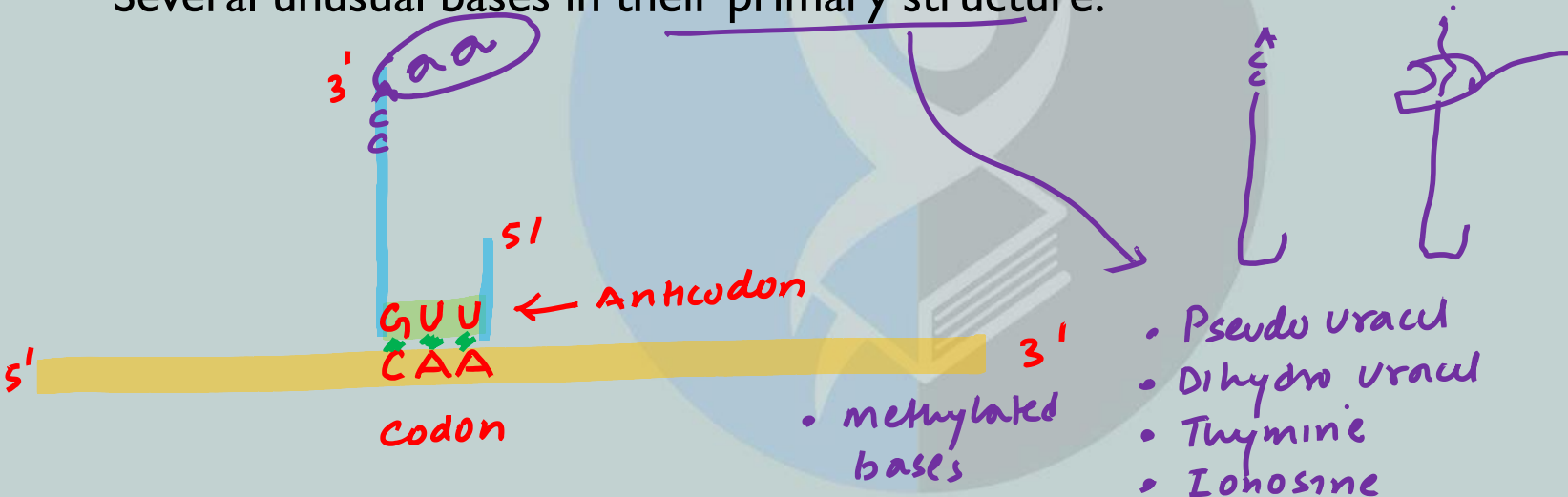
Drosophila

XIST, AIR

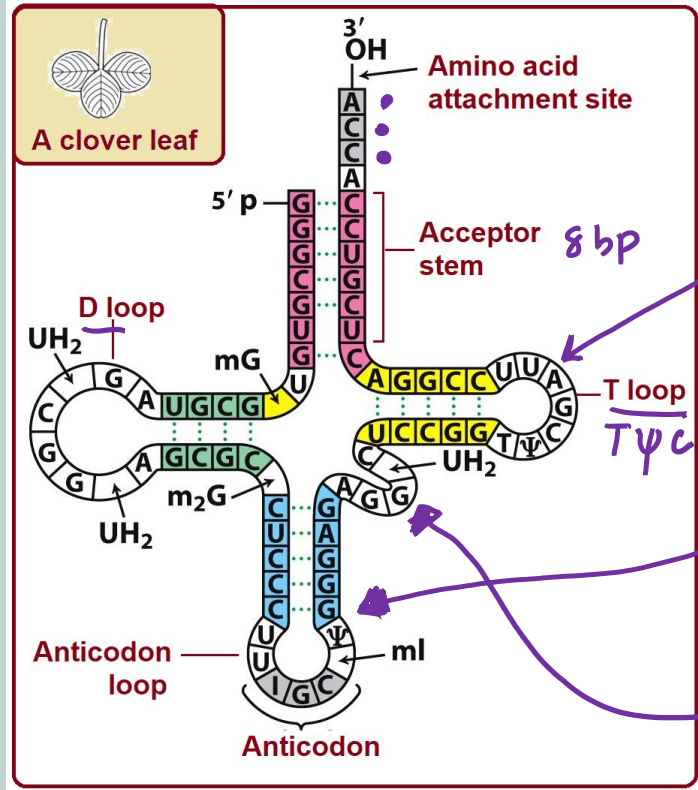


tRNAs : Adaptors between Codons and Amino Acids

- 75 and 95 ribonucleotides in length.
- All tRNAs end at the 3' terminus with the sequence 5'-CCA-3'
- (co-transcription/post transcriptional addition)
- Several unusual bases in their primary structure.



tRNAs Share a Common Secondary Structure That Resembles a Cloverleaf : Stabilized by H-bonding



The acceptor arm (CCA): 8bp stem and CCA sequence - 3'
 ↳ addition of amino acid

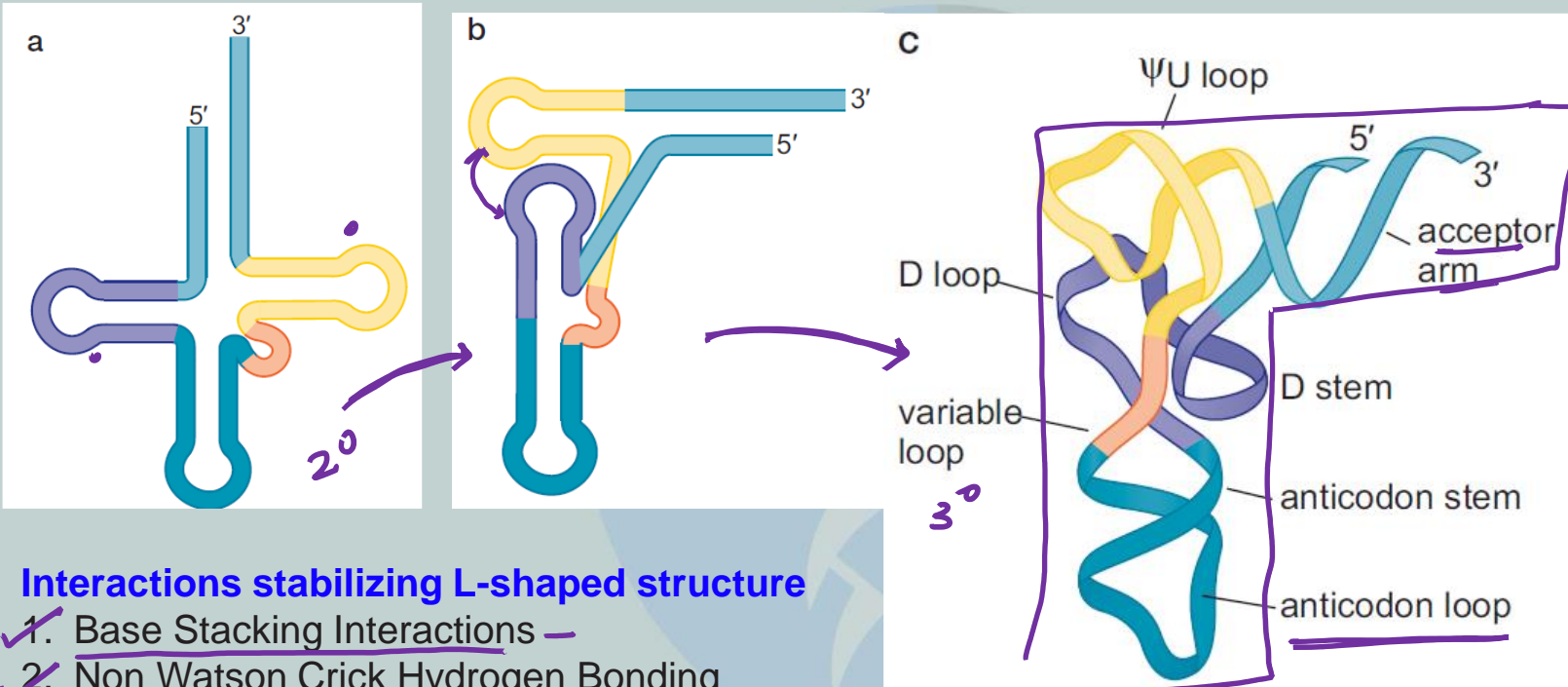
Pseudo Uracil (TΨC) arm: T, Pseudo uracil

Di hydro Uracil (D) arm : Dihydro uracil

Anticodon arm: Stem followed and loop
 NN^UXXX^RNN
 Anticodon

Variable loop (3-21):

tRNAs Have an L-Shaped Three-Dimensional Structure (3° str)

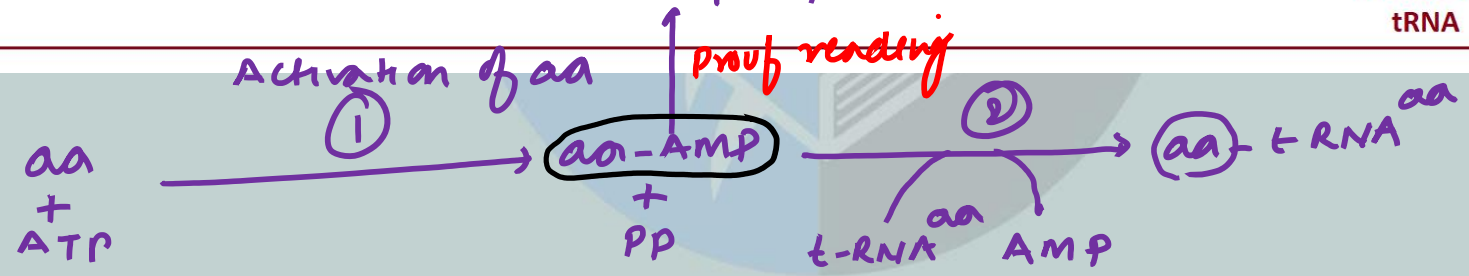
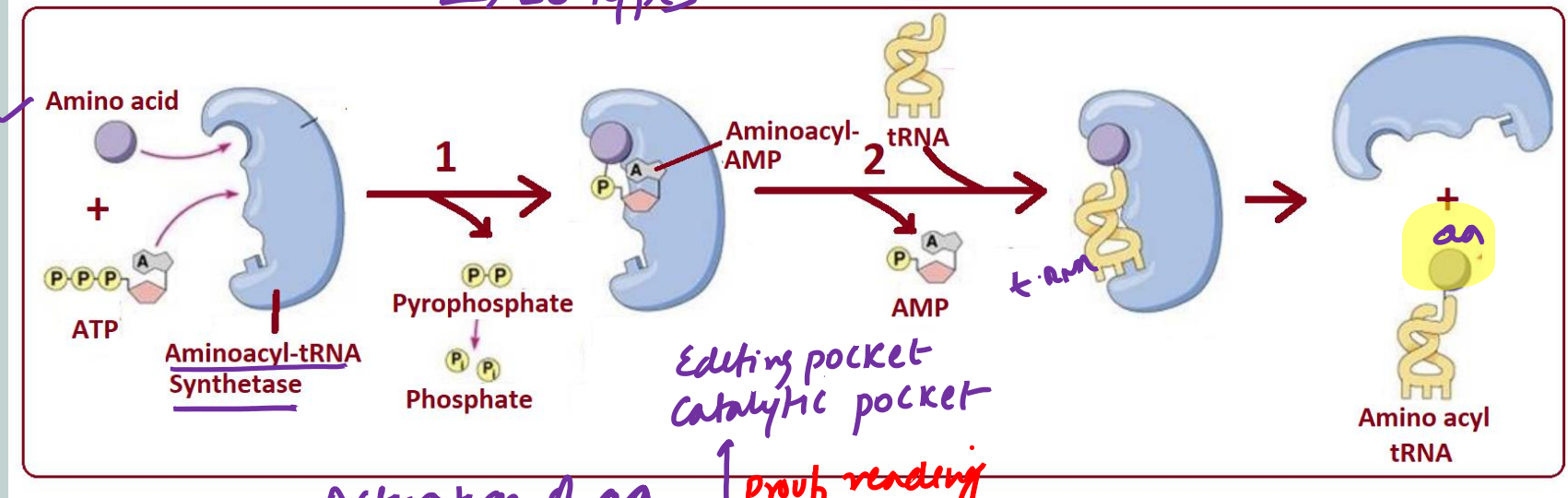


Interactions stabilizing L-shaped structure

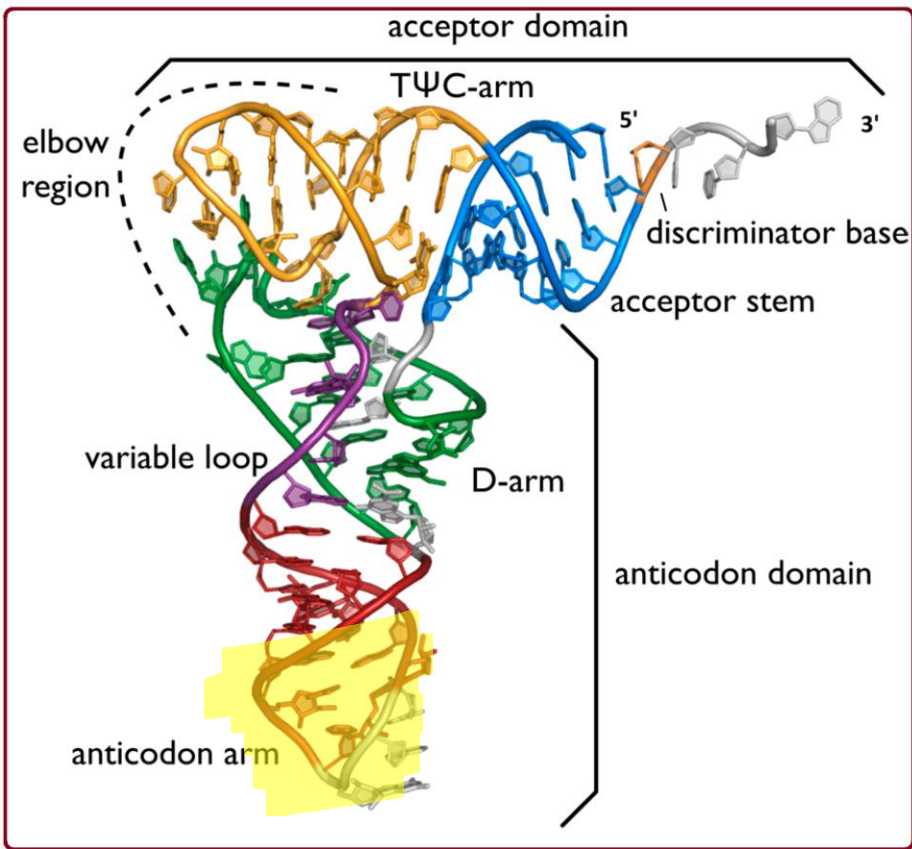
- ✓ 1. Base Stacking Interactions —
- ✓ 2. Non Watson Crick Hydrogen Bonding
- ✓ 3. Interaction among Bases and sugar phosphate backbone

Aminoacyl-tRNA Synthetases Charge tRNAs in Two Steps

↳ 20 types



tRNA Synthetases Recognize Unique Structural Features of Cognate



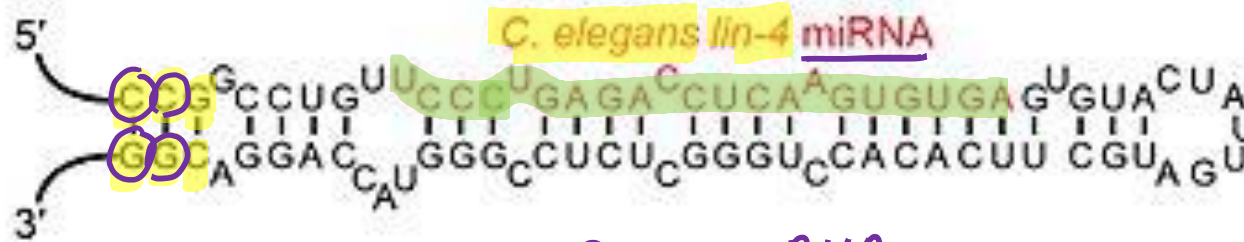
Second genetic code

1. Anticodon loop
2. Acceptor arm (discriminator base)

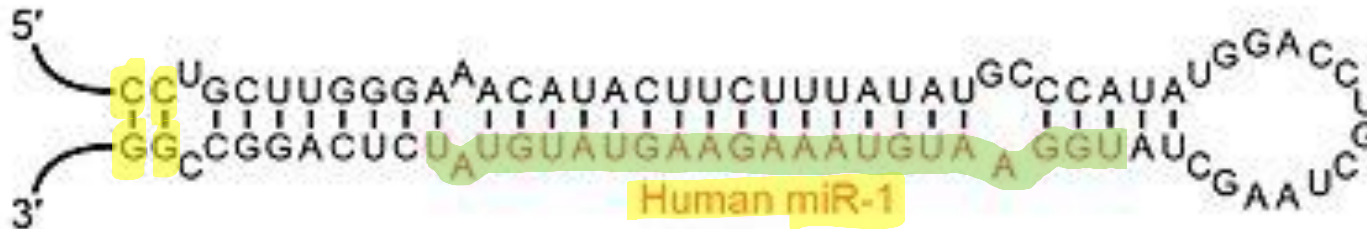
Interfering RNA (RNAi):

- 21-23 nucleotides ~~long~~ small RNA
- ✓ • miRNA (Micro RNA)- Genes in our genome for mi-RNA
- ✓ • siRNA (small interfering-RNA): Origin from double stranded exogenous RNA (viruses)

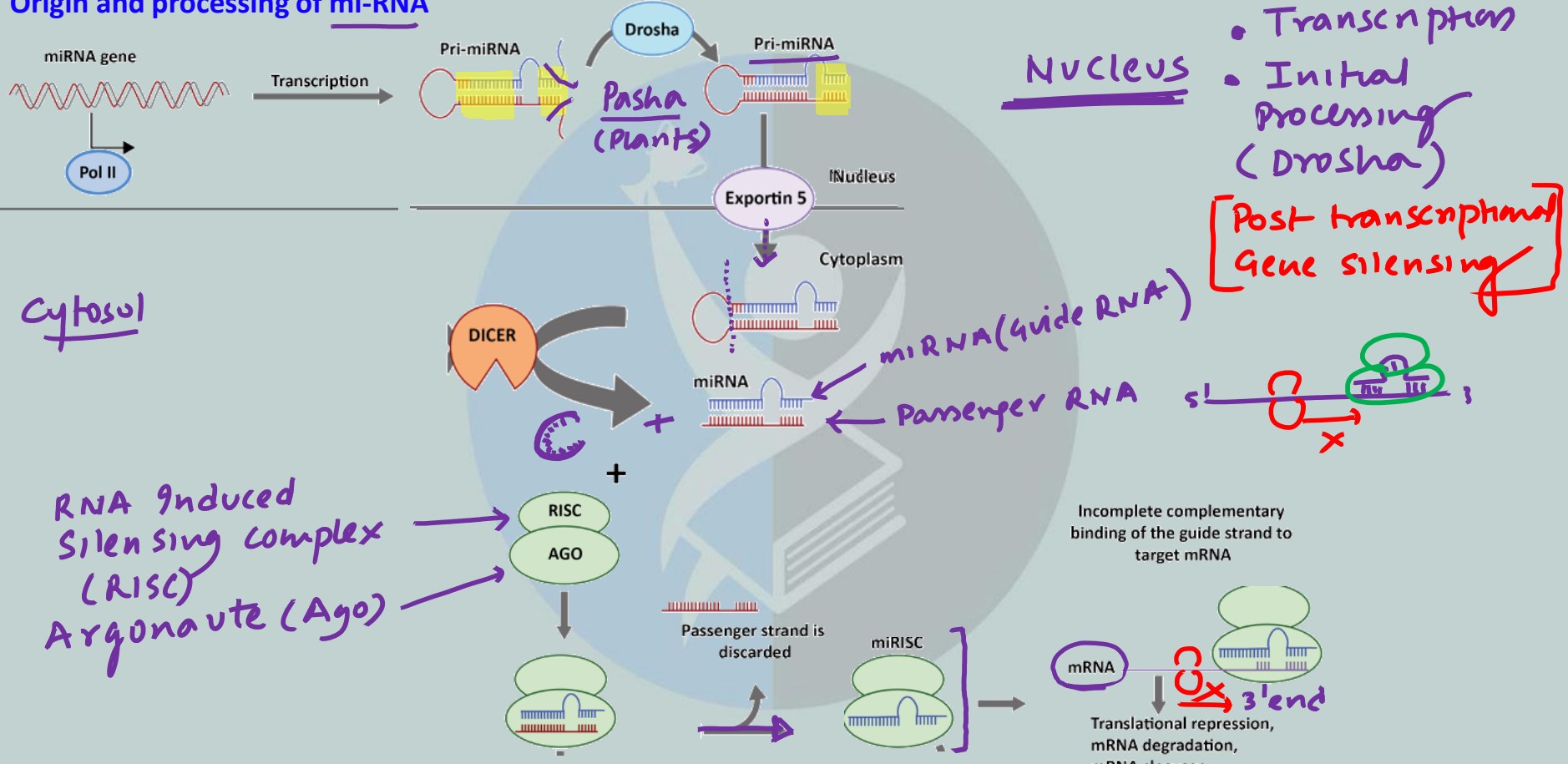
Transcribed by RNA poly II



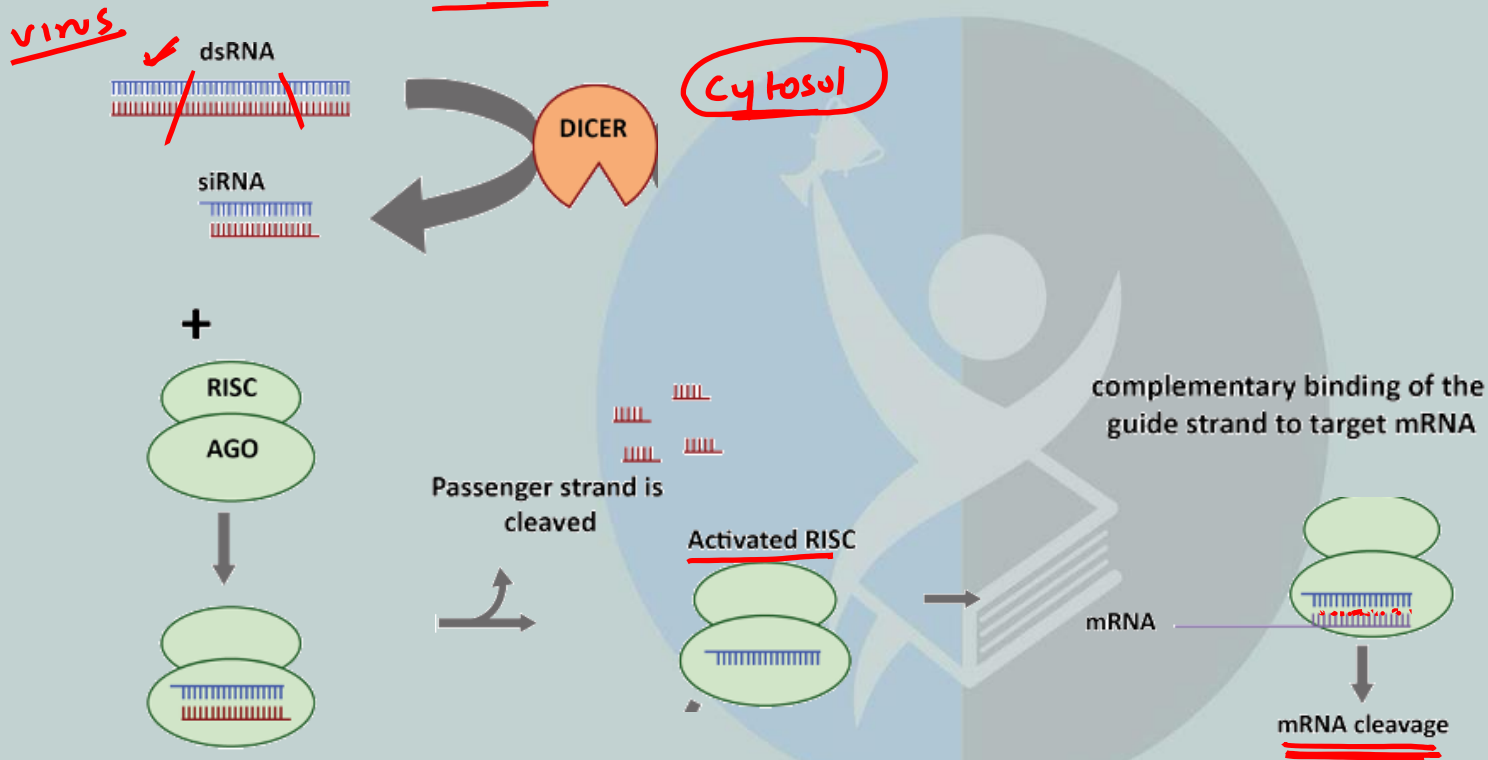
Pre - miRNA

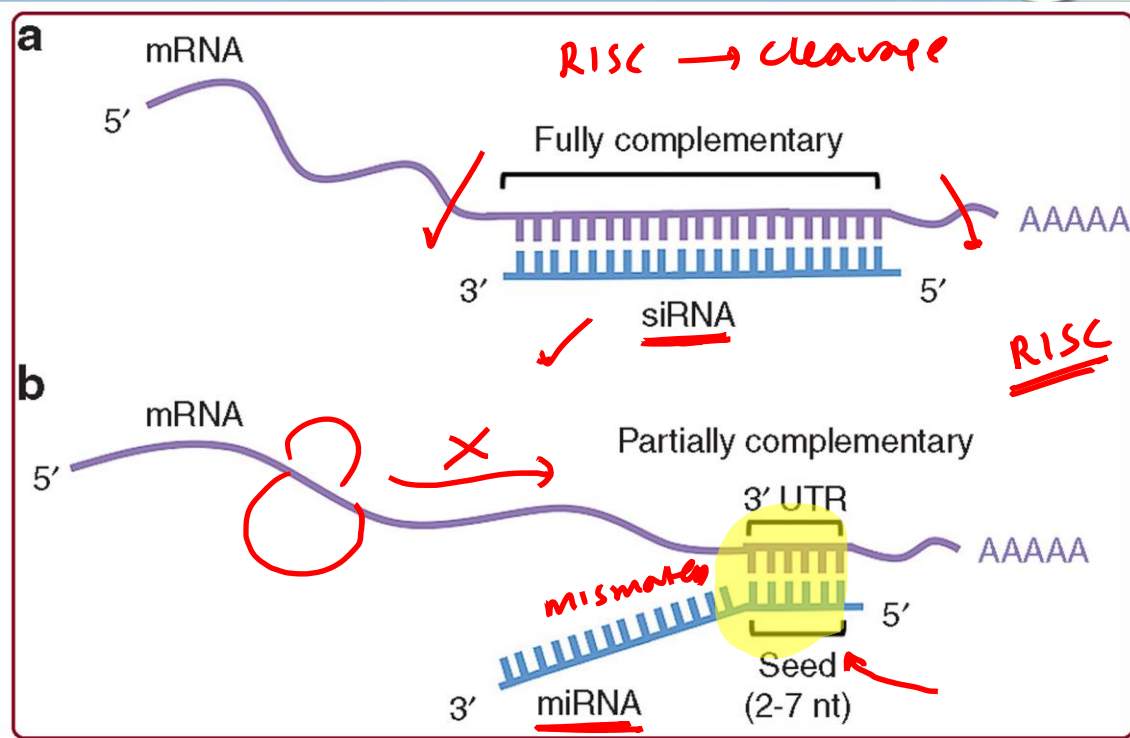


Origin and processing of mi-RNA



Origin and processing of Si-RNA






siRNA or miRNA inhibit expression of homologous target genes in 3 ways:

- Destruction of the target mRNA (if perfect complementary) eg siRNA
- Inhibition of translation from the target mRNA (if have some mismatch) eg miRNA
- Induce chromatin modifications within the target gene and thereby silence its transcription

RITS

RNA induced
transcriptional
silencing

	siRNA	miRNA
Prior to Dicer processing	Double-stranded RNA that contains 30 to over 100 nucleotides	Precursor miRNA (pre-miRNA) that contains 70-100 nucleotides with interspersed mismatches and hairpin structure
Structure	21-23 nucleotide RNA duplex with 2 nucleotides 3' overhang 	19-25 nucleotide RNA duplex with 2 nucleotides 3' overhang
Complementary	Fully complementary to mRNA	Partially complementary to mRNA, typically targeting the 3' untranslated region of mRNA
mRNA target	One	Multiple (could be over 100 at the same time)
Action	Generated by transcripts of the regions on which they act, thus acting in cis	Act like traditional trans-acting regulators: they are encoded by a gene but act on other genes
Mechanism of gene regulation	Endonucleolytic cleavage of mRNA	Translational repression, Rare degradation of mRNA Endonucleolytic cleavage of mRNA



Thank you

Learn From India's Best Educators
India's **No.1** EdTech Company for Graduates
& Post Graduates Examination.

➡ Download **IFAS** App Now



ANDROID



IOS



WINDOWS



Harshada Sharma

Earned: ₹25,500

"As is widely recognized, IFAS stands as India's leading institute for CSIR NET examination preparation. My Personal philosophy has always revolved around helping others and I've had the pleasure of introducing the IFAS Online Course to 12 of my classmates and juniors, which in turn allowed me to earn 25,500 rupees. You too can join in and refer your friends, allowing you to earn a 5% rewards based on the fees each of your referred friends Pay"



Step – 01 Invite Friends

Share your referral code or Link with your friends.

Step – 02 Earn Cashback

On Every Purchase made using your referral code, you earn 5% instant cashback in your bank account.

Step – 03 Refer More, Earn More

Keep referring as many friends as you can and keep **Earning** cash as much as you can



Friendship is Sharing, Caring, Giving & Helping Each Other...!